Seroprevalence of Toxoplasma gondii infection in Camels (Camelus dromedaries) in & around Bahawalpur

Seroprevalence of *Toxoplasma gondii* infection in Camels (Camelus dromedaries) in & around Bahawalpur

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TO,

The Controller of Examinations,
University of Veterinary & animal sciences,
Lahore.

We, the supervisory committee, certify that the contents and form of the thesis, submitted by **Mr. Umer Naveed Chaudhary Regd. No 2005-VA-190** have been found satisfactory and recommend that it be processed for the evaluation by the external examination (s) for award of the degree.

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IN THE NAME OF ALLAH,
THE COMPASSIONATE, THE MERCIFUL

All Praises and thanks are for

Almighty ALLAH,

The source of all knowledge and wisdom
Endowed to mankind, who guides us in
Darkness and helps us in difficulties
And all respects are for his last
Holy prophet

HAZRAT MUHAMMAD
(Peace Be Upon Him)

Who enabled us to recognize our creator

WISDOM IS THE PART AND PARCEL

OF MY RELIGION,

KNOWLEDGE MY DRESS,

PATIENCE MY WEAPON,

FAITH MY DIET,

AND SINCERITY

MY COMPANION

(HADIS-E-NABVI)

DEDICATION

To my dear parents, sisters & wife

Who dedicated their lives for me

Dearest mother and father & sisters,

I know and understand, you actually gave me

More than one life, my own and yours.

So much of what I have become is just because of you.

I can only show you my extreme appreciation for your

Support by being true to all the ideals and values that

You tried to teach me,

Thanks your forever for standing by me.

Today my definition of happiness is being with you

Umer Naveed Chaudhary

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UMER NAVEED CH

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INTRODUCTION

CHAPTER 1
INTRODUCTION

Toxoplasma gondii is an intracellular protozoan parasite (Smith 1995) which infects humans as well as wide variety of mammals and birds (Hill *et al.,* 1998). Toxoplasmosis is found throughout the world and tends to be more prevalent in tropical climates (Dubey 1999). The organism was first discovered by Nicolle and Manceaux (1908) as a tissue parasite of gondii (an African rodent), and Darling found it in Man (Subash 1990). The infection has been confirmed in some 200 species of mammals including man and in domestic / wild felines, which are the only definitive host (Pedro *et al.,* 2003).

Toxoplasma gondii exhibits a predator-prey type life cycle having two phases (Torada 2001). The intestinal phase of the infection occurs only in felines when the cysts are ingested by a cat and multiply asexually by mergony and then sexually by gametogony and produce oocyst (Pedro *et al.,* 2003). The tissue phases occur in intermediate host, when sporulated oocyst ingests and penetrate through intestinal wall and spread by haematogenous route. This stage is called tachyzoite. The tachyzoite infect tissues throughout the body and replicate intracellular until the cell burst and cause tissue necrosis. As a result there is formation of tissue cysts containing bradyzoites. Tachyzoites and bradyzoites in extra intestinal tissue including muscles, liver, lungs, spleen and brain (Urquhart *et al.,* 2000).

The source of transmission is the ingestion of vegetables, fruits, water, soil, food contaminated by cat faeces, raw or undercooked meat. Flies and cockroaches may act as a

mechanical carrier to transfer oocysts to different varieties of foods. Other sources include transplcental transmission, from mother to the offspring through milk, transplantation of organs, transfusion of blood and venereal transmission (Pedro *et, al.,* 2003).

Toxoplasma gondii can cause severe acquired infection in animals and human beings, which may be localized or generalized. Lymphadenitis is the most frequently observed clinical sign (deep cervical nodes). Other signs include fever, retinochoroiditis, uveitis, malaise, muscle pain, muscle fatigue, sore throat, headache, hepatitis, myocarditis and pneumonia. Encephalitis is an important sign of *Toxoplasma* in later stages. During the 1980,s toxoplasmic encephalitis in humans emerged as a common complication associated with AIDS (Subash 1990).

As for as congenital infection is concerned, animals and pregnant women develop the most serious side effects leading to spontaneous abortion, still birth, birth defects, mummification, neonatal losses or fetal abnormalities including microcephalya, hydrocephalya, brain calcifications, psychomotor & mental retardation. The mechanism of vertical transmission is not yet understood (Remington *et al.,* 1995).

The disease has zoonotic importance in human population and depending upon the geographic location. In human 15 – 80 % population is infected with *toxoplasmosis.* Approximately 500 million populations are estimated to have antibodies to *T. gondii* infection (Subash.1990). Study has shown that between 16% to 40% of the human population in North America and Great Britain, 50% to 80% of the populations in Europe

and Latin America have antibodies of *T. gondii,* indicating that they have got infection at some time (Pedro *et, al.,* 2003).

Serodiagnosis has been a reliable tool to diagnose *Toxoplasma* infection in both man and animals, using various serological tests, such as indirect haemagglutination, indirect immunoflorescent technique, and Enzyme linked immunosorbant assay and latex agglutination test (Ahmed *et al.,* 1983). Due to increasing risk of public health by ingestion of contaminated meat, toxoplasmosis has becomes extremely important zoonotic disease.

Camel meat is commonly being consumed, so that camel is the most vulnerable to the exposure of toxoplasmosis which may become the potential source of infection for the consumers; so for as no literature could be traced relating to the investigation of toxoplasmosis in Pakistan in camels. Therefore, keeping in view the importance of disease, study on seroprevalance of *Toxoplasma gondii* in camels were carried out, which would be helpful to adopt the control measures against the diseases in humans.

REVIEW OF LITERATURE

CHAPTER 2
REVIEW OF LITERATURE

Ridi *et al,* (1990) conducted study to estimate the prevalence of Toxoplasma antibodies among farm animals in Zigzags slaughterhouses using indirect haemagglutination test (IHAT) and to evaluate the occupational exposure to infected animals and meat among abattoir workers. 25% of the tested animals were seropositive. 19.2% of the exposed persons showed positive reactions while in a control group it was 12% with statistically insignificant difference.

Hagemoser *et al,* (1990) examined 6-year-old camel having history of dyspnoea of unknown duration. Approximately 24 L of turbid fluid was drained from the pleural cavity. Numerous Toxoplasma gondii tachyzoites were found in macrophages in smears of the pleural fluid. High titers (1:20,000) of T gondii antibodies were found in pleural fluid.

Elamin *et. al.,* (1991) tested a total of 482 serum samples from pastoral camels in the Butana plains, mid-Eastern Sudan, for Toxoplasma antibodies by the latex agglutination test (LAT). 67% of the camels were seroreactive. The prevalence rate of seroreactivity increased significantly with age (P less than 0.01) and was highest among camels aged over 7 years (74.2%). The prevalence rate of seropositivity decreased proportionally with the level of serum dilution. At dilutions of 1:32 and above, the prevalence rate was 25.9%. There were no sex-linked differences in seroreactivity. This study suggests widespread infection with T. gondii among pastoral camels, a finding that warrants a closer look into the possible ways infection is acquired by camels in their arid

environment, its economic impact, as well as its public health significance, especially among the nomads who consume cameline milk and liver raw.

Dubey *et al,* (1992) tested serum samples from 283 llamas (Lama glama) from Oregon, Washington State and Idaho for antibodies to *Toxoplasma gondii* using the modified agglutination test. Antibodies were found in 95 (33.5%) llamas. Percent seropositivity in serum dilutions of 1:25, 1:50, 1:500, and 1:5000 was 9.5%, 18.3%, 4.9%, and 0.7%, respectively.

Lings *et al,* (1994) examined 217 slaughter men and a control group of 113 greenhouse workers were investigated for the prevalence of serum antibodies to Toxoplasma gondii, Campylobacter jejuni (IgA and IgG), Yersinia enterocolitica types 3 and 9, Yersinia pseudotuberculosis types I, II, III, IV, and V, Salmonella typhi, Salmonella paratyphi, Salmonella enteritidis, Salmonella typhimurium, and Borrelia burgdorferi. No significant differences were found concerning either frequency of positive tests or magnitude of titers. The prevalence of toxoplasma antibodies was remarkably high in both groups.

Chaudhary *et Al,* (1996) reported 18% incidence of Toxoplasmosis in racing camels. A significant ($P< 0.05$) decrease of the WBCs and an increase in eosinophilic counts was observed in camels positive to Toxoplasmosis. The blood chemistry parameters (alkaline posphatase, alanine aminotransferase, creatinine phosphokinase, lactic dehydrogenase, urea, total protein, calcium, creatinine, posphorus, iron, and albumin) were not influenced in camels bearing antibodies against Toxoplasma.

Fortier *et at,* (1996) reported that tachyzoite-bradyzoite interconversion is one characteristic feature of Toxoplasma gondii. Although highly similar in structure, tachyzoite and bradyzoite differ by the relative amount of certain organelles and by specific surface or cytoplasmic molecules. Differences in structure and contents also exist between parasitophorous vacuoles and cysts. Using stage specific markers, it was shown the quickness of stage switching in vivo as well as in vitro, together with the occurrence of intermediate stages. Regulatory mechanisms of interconversion remain unknown. However, stress or inhibition of the mitochondrial metabolism of the parasite trigger bradyzoite formation.

Ibrahim *et al,* (1997) reported that *Toxoplasma gondii* is one of the important zoonotic parasite of worldwide zoological and geographical distribution. In this paper, Toxoplasma antibodies were investigated among workers (21) and slaughtered animals (258) in Tanta abattoir. The indirect haemagglutination test (IHAT) was positive among workers (52.4%) at titers 1/64, 1/256, 1/512 and slaughtered animals (44.1%), at titers > 1:64. The indirect fluorescent antibody test (IFAT done for animals only) was positive (48.8%) at titers 1/40. Histopathological study on infected prescapular lymph nodes of the examined animals showed different clinical pictures, which coincided with the serological results. It was concluded that meat should not be consumed uncooked or undercooked.

Hussein *et al,* (1998) examined Serum samples from 227 Saudi Arabian camels for *Toxoplasma gondii* antibodies by the indirect haemagglutination test, using a microtitre technique. Agglutinations (greater than 2+) occurring at 1:64 dilution were considered

positive. A total of 36 (16%) camels were serologically positive for toxoplasmosis, giving titres ranging between 1:64 and 1:8192. The prevalence was much higher in female compared to male camels and in adults compared to young individuals.

Hilali *et al,* (1998) examined sera from camels from Egypt were examined by the direct agglutination tests incorporating mercaptoethanol for antibodies to *Neospora caninum* and *Toxoplasma gondii*. Antibodies to *N. caninum* were found in 6 of 161 camels in titers of 1:40 (2 camels) and 1:80, 1:160, 1:640, and 1:1280 in 1 camel each, using *N. caninum* formalin preserved whole tachyzoites as antigen. Antibodies to *T. gondii* were found in 17.4% of 166 camels in titers of 1:25 (3 camels), 1:50 (18 camels). and > 1:500 (8 camels) using *T. gondii* tachyzoites. All 6 camels with *N. caninum* antibodies had no *T.gondii* antibodies in 1:4 dilution of serum, indicating specificity of the reaction. This is the first report of N. caninum prevalence in Egypt.

Lyons *et. al.*, (2001) reported that *Toxoplasma gondii* exists as 2 life-cycle forms in intermediate hosts. The rapidly dividing tachyzoites responsible for acute disease, present in the first 14 days of infection, give rise to slowly dividing bradyzoites that reside in tissue cysts. Reactivation of disease is associated with conversion of bradyzoites to tachyzoites. A sensitive method for detection and assessment of the number of each life-cycle stage would be useful for following these events. Herein we describe the construction and validation of a plasmid (pSWITCH) containing a polycompetitor construct (SWITCH) for use in competitive reverse transcriptase-PCR (cRT-PCR). pSWITCH contains competitors for SAG2A and LDH2 genes, which are exclusively expressed by tachyzoite and bradyzoite stages respectively, and for beta-tubulin, a gene

expressed by both stages. Using cRT-PCR, samples can first be accurately normalized for expression of the housekeeping gene, beta-tubulin and then the relative levels of SAG2A and LDH2 expression compared to follow stage conversion. The abundance of transcripts for other genes of interest can then be followed during this process as demonstrated here for the SAG2-related family of genes. This technique offers a powerful tool for studying the processes involved in tachyzoite and bradyzoite interconversion.

Tomavo *et al,* (2001) studied that *Toxoplasma gondii* has the ability to switch between a rapidly replicating tachyzoite and a slowly dividing encysted bradyzoite within its intermediate hosts. It is likely that in vivo, the tachyzoites differentiate into encysted bradyzoites in response to the immune system attack during disease progression. As part of a developmental strategy and, in order to survive within infected hosts, *T. gondii* tachyzoites undergo profound metabolic and morphological changes by differentiating into encysted bradyzoites. Bradyzoites are characterised by their resistance to both the immune system and chemotherapy. The stimulus that triggers Toxoplasma encystation and the molecular mechanisms triggering the switch from tachyzoite to bradyzoite remain unknown. It is very important to elucidate these mechanisms since bradyzoites within tissue cysts are not only the source of infection transmitted from domestic animals to humans, but can also be converted into tachyzoites that are the cause of fatal toxoplasmic encephalitis in acquired immunodeficiency syndrome patients. In this review, I focus on recent efforts towards the characterisation of genes that encode several stage-specific isoenzymes. The picture emerging from these studies is that stage-specific expression of isoenyzmes having different biochemical properties accompanies the interconversion of tachyzoite into bradyzoite, and vice versa. It can be hypothesised that the difference

found between these enzymatic activities may be instrumental in maintaining some major parasitic metabolisms such as glycolysis in pace with the stage-specific requirements of carbohydrate or polysaccharide biosynthesis.

Lyons *et al,* (2002) reported that *Toxoplasma gondii* infection undergoes stage conversion between the rapidly dividing tachyzoite in the intermediate host, which is responsible for acute toxoplasmosis and the slowly replicating, encysted bradyzoite stage. This process of tachyzoite-bradyzoite interconversion is central to the pathogenesis and longevity of infection. Recent research has identified several stage-specific genes and proteins. However, despite recent advances in the understanding of Toxoplasma cell biology, more research is necessary to elucidate the complex events occurring during tachyzoite-bradyzoite interconversion. Here, a brief summary of this process is provided and a new method to characterize gene expression during interconversion is introduced.

Khalil *et al,* (2002) tested a total of 153 serum samples from one-humped camels (*Camelus dromedarius*) and 45 serum samples from their drivers and herdsmen from Butana plains,Northern Kordofan and Southern Kordofan for *Toxoplasma gondii* antibodies by the Latex Agglutination Test (LAT).The seroreactivity of the camels was 22.2%,while 53.3% of the camels' drivers were positive by the same test. There was a relationship between the prevalence in camels and their drivers only in Butana plains ($P<0.05$). No significant association was found between age in camels' drivers and sero-reactivity ($P>0.05$). The study suggests a widespread infection with *Toxoplasma gondii* among camels and their drivers especially those who consume and depend on cameline unboiled milk and raw liver.

Abu-Zeid **(2002)** used ELISA for detection of antibodies against the immunodominant surface antigen 1 (SAG1, synonymous P30) of *Toxoplasma gondii*, and peroxidase-conjugated protein G was used instead of commercially unavailable enzyme-conjugated anti-dromedary antibody. A latex agglutination test was employed to select 20 seronegative control animals, and peroxidase-conjugated protein A was used for comparison with protein G. The overall seroprevalence rate was 31.4%; males had to some extent higher seropositive rate than females ($P = 0.077$). Seropositive camels sampled in winter had significantly higher-antibody levels than those sampled in summer ($P < 0.01$). ELISA values using protein G and protein A conjugates were significantly correlated (Spearman's rho = 0.797; $n = 185$; $P < 0.001$). The results were discussed.

Sroka ***et al,*** **(2003)** reported that abattoir workers are occupationally exposed to Toxoplasma gondii by the contact with raw meat. 107 abattoir workers from the Meat Factory in Lublin were examined for the presence of anti-Toxoplasma antibodies. 61 blood donors were also tested as the reference group. Sera from workers and referents were tested by direct agglutination with 2-mercaptoethanol (DA-2ME), and also by ELFA IgG and ELFA IgM tests. In the workers group, out of 107 tested sera, 70 were found positive (65.4%). The highest percentage of seropositive results was found in the Cured Meat Division 76.2%. In the Meat Production Division 66.6% of seropositive results were found, and in the Slaughter Division 46.1%. Three persons with the presence of IgM antibodies were found in the Cured Meat Division. In the reference group, 34 out of 61 sera (55.7%) were positive. The difference in seropositivity between Cured Meat Division workers and reference group was statistically significant ($p < 0.05$). The high percentage of seropositive reactions among the workers of Cured Meat Division and the

presence of persons in early stage of invasion suggest an increased risk of exposure to T. gondii in this section.

Coppin *et al,* (2003) studied that during infection in the intermediate host, *T. gondii* undergoes stage conversion between the rapidly replicating tachyzoite that is responsible for acute toxoplasmosis and the dormant or slowly dividing encysted bradyzoite. The tachyzoite-bradyzoite interconversion is central to the pathogenic process and is associated with the life-threatening recrudescence of infection observed in immunocompromised patients such as those suffering from AIDS. In chronic infections, the bradyzoites are located within tissue cysts found predominantly in brain and muscles. The tissue cyst is enclosed by a wall containing specific lectin binding sugars while the bradyzoites have accumulated large amounts of the storage polysaccharide of glucose, amylopectin. Our recent findings have identified several genes and proteins associated with amylopectin synthesis or degradation and glucose metabolism, including different isoforms of certain glycolytic enzymes, which are stage-specifically expressed during tachyzoite-bradyzoite interconversion. Here, we will discuss how the genes and enzymes involved in carbohydrate metabolisms are used as molecular and biochemical tools for the elucidation of molecular mechanisms controlling T. gondii stage interconversion and cyst formation.

Marco Saravia *et al,* (2004) determined the seroprevalence of *Toxoplasma gondii* in adult female llamas in two flocks of the Rural Alliance farm in the province of Melgar, Puno. A total of 157 blood samples were collected and analyzed using the indirect immunofluorescence test. The results indicated that 10.2 ± 4.7% (16/157) of llamas had

antibodies against T. gondii. One flock had 13.3 ± 9.8% (6/45) seroprevalence and the other one had 8.9 ± 5.3% (10/112); however, no significant statistical differences were found between flocks or age groups. The results showed a relatively low seroprevalence to T. gondii in relation to other studies in the country.

Gualberto *et al,* (2004) studied the seroprevalence of *Toxoplasma gondii* in female llamas from two farms located in the Melgar province, Puno. A total of 284 blood samples were tested using the indirect immunofluorescence test (IFAT) to detect antibodies against T. gondii. The results showed that 47.5 + 5.8% (135/284) of the samples had antibodies and they increased with age. The seroprevalence from birth till 2 years, 2-4 years, >4-6 years, and >6 years was 33.8 ± 11.0, 51.2 ± 10.8, 56.8 ± 14.6, and 50.6 ± 10.5%, respectively. There were significant differences ($p<0.05$) in the frecuency of female reactors between farms.

Wolf *et al,* (2005) collected serum samples from a total of 871 South American Camelids (SAC: Lama glama, Lama pacos, Lama vicugna) from two farms in Peru and from 32 SAC of a farm in central Germany and these were examined for antibodies against *N. caninum* and *Toxoplasma gondii*. Based on the recognition of specific bands in the immunoblot, sera of SAC from Peru were differentiated into *N. caninum*-positive (n = 18) and *T. gondii*-positive (n = 30) samples and into samples negative or inconclusive for both parasites. Using the immunoblot results as the reference, a modified version of the p38-ELISA and the IFAT were evaluated for detecting *N. caninum* antibodies in SAC sera. Applying a cut-off as determined by two graph-receivers operating characteristic analysis both, the ELISA and the IFAT, exhibited a sensitivity and specificity of about

95% in the SAC sera from Peru. Serological testing confirmed that SAC may become infected with *N. caninum* under field conditions in Peru. In addition to alpacas and llamas also 114 wild living vicunas had been examined for antibodies against N. caninum. However, only the alpacas and llamas but no vicunas were found N. caninum-positive. In contrast, T. gondii-seropositive animals were detected in all three SAC species. The lack of N. caninum-seropositive vicunas indicates that in the study area in Peru wild canids might not serve as definitive hosts of N. caninum while for T. gondii a life cycle including wild felids is likely. On the German farm no N. caninum- but only T. gondii-seropositive SAC (n = 14) were detected. The seroprevalence of T. gondii infection was significantly higher in adult SAC (alpacas in Peru, llamas in Germany) than in crias (i.e. < 12 months old foals) indicating that the predominant route of infection is post natal.

Chávez-Velásquez *et al,* (2005) reported that *Toxoplasma gondii* infection in adult llamas (Lama glama) and vicunas (Vicugna vicugna) in the Peruvian Andean region, for which to date no information has been available. Serum samples from 43 llamas (L. glama) and 200 vicunas were tested by IFAT detecting titres of 1:50 or higher in 55.8% (33.9-70.9%) and 5.5% (2.8-9.6%), respectively. IFAT titres ranged from 1:50 to 1:6400. In order to avoid cross reactions with closely related coccidian parasites and to confirm the existence of T. gondii specific antibodies, IFAT positive sera from both ruminant species were also analysed by western blot. T. gondii specific antigens were recognised by IFAT positive sera, although different IFAT cut-off points could be selected for llamas (1:200) and vicunas (1:50) meaning seroprevalence of 44.2% (29.1-60.1%) and 5.5% (2.8-9.6%), respectively. Based on the frequency and intensity of tachyzoite antigen recognition, at least three immunodominant antigens with apparent molecular weights of

22-24, 30, and 38-40 kDa were detected, together with other minor protein fractions located in the 18-73 kDa range. This study documents for the first time the presence of T. gondii infection and reports the target T. gondii antigens in adult llamas and vicunas in Peru

Sadrebazzaz *et al,* (2005) used one hundred twenty camels were blood-sampled and to evaluate serological screening for *Neospora caninum* and *Toxoplasma gondii* infection by indirect fluorescent antibody test (IFAT) in Mashhad, Iran, during years 2004–2005. Of the 120 camels, antibodies to *N. caninum* were found in three in titers of 1:20 and in four in titers of 1:40 using whole *N. caninum* tachyzoites as IFAT slide. Antibodies to *T. gondii* were found in three camels in titers 1:20 and in two camels in titers 1:40 using whole *T. gondii* tachyzoites as IFAT slide.

Yousif *et al,* (2005) reported that camels exposure to *toxoplasmosis* is mainly through ingesting the *oocysts* from the environment and therefore genotyping of *T. gondii* in camels is a good indicator of prevalent genotypes of *T. gondii* in their environment. meat and blood samples were collected from Al khazna Tannery in Abu Dhabi from 58 camels. Serological diagnosis was done using the modified agglutination test (MAT) and P30 ELISA, DNA extraction from meat of serologically positive camels and genotyping of isolates was done by nested PCR at the SAG2 locus followed by restriction fragment length polymorphism (RFLP). Thirteen sera samples were found positive in both serological tests, whereas 8 only by MAT and 3 only by P30 ELISA. Therefore the *Toxoplasma gondii* sero-positive rate among slaughtered camels from Abu Dhabi ranged between 22.4 to 41.4%. Such high prevalence rate is in agreement with previously

reported sero-prevalence rates of greater than 30% among camels in UAE. Most strains typed were genotype I or II, none was type III. The significance of the results with regard to possible impact on humans is discussed.

Sawadogo *et al,* (2005) studied 261 sera from animals intended for consumption in Marrakech were subjected to the Toxoplasma ELISA based serology test for the detection of anti-T. gondii specific IgG confirming a past infection. Of the total tested 72 (27.6%) sera were positive for IgG. This result shows that the seroprevalence approaches the world average and is similar to what is found in other cities of Morocco. This has prompted us to investigate other animal species in the region in order to evaluate the degree of contamination by this parasite as well as the potential risk incurred on consumption of their meat.

Hughes *et al,* (2005) reported that *Neospora caninum* and *Toxoplasma gondii* are closely related intracellular protozoan parasites.Little is known about the extent of *Neospora/Toxoplasma* co-infection in naturally infected populations of animals. Using nested PCR techniques, based on primers from the Nc5 region of *N. caninum* and SAG1 for *T. gondii*, the prevalence of *N. caninum* and its co-infection with *T. gondii* were investigated in populations of *Mus domesticus*, *Rattus norvegicus* and aborted lambs (*Ovis aries*). A low frequency of infection with *N. caninum* was detected in the *Mus domesticus* (3%) and *Rattus norvegicus* (4·4%) populations. A relatively high frequency of infection with *N. caninum* was detected in the brains of aborted lambs (18·9%). There was no significant relationship between *N. caninum* and *T. gondii* co-infection. Investigation of the tissue distribution of *Neospora*, in aborted lambs, showed that

Neospora could not be detected in tissues other than brain and this was in contrast to *Toxoplasma* where the parasite could be frequently detected in a range of tissues.

Tibary *et al,* (2006) reported that reproductive losses in camelids are due to infertility, pregnancy loss, udder diseases and neonatal mortality caused by a variety of infectious diseases. Uterine infection and abortion represent the major complaint in camelid veterinary practice. The major infectious organisms in endometritis and metritis are E. coli and Streptococcus equi subspecies zooepidemicus. Abortion rates due to infectious diseases vary from 10% to more than 70% in some areas. Leptospirosis, toxoplasmosis and chlamydiosis have been diagnosed as the major causes of abortion in llamas and alpacas. In camels, brucellosis and trypanosomiasis represent the major causes of infectious abortion in the Middle East and Africa. Mastitis is rare in South American camelids. The prevalence of subclinical udder infection in camels can reach very high proportions in dairy camels. Udder infections are primarily due to Streptococcus agalactiae and Staphylococcus aureus. Neonatal mortality is primarily due to diarrhea following failure of passive transfer and exposure to E. coli, rotavirus, coronavirus, Coccidia and Salmonella. This paper reviews the etio-pathogenesis of these causes of reproductive losses, as well as the major risk factors and strategies to prevent their occurrence.

Karimi (2006) tested blood samples from 600 native camels (*Camelus dromedaries*) for *toxoplasma gondii* antibodies by the indirect flourecent antibodies test (IFA). Of these samples 36 (6%) were seroreactivity positive for *T. gondii* antibodies. The prevalence rate of seroreactivity increased with age and was highest among camels aged over 6 years

(9.58%). The prevalence was higher in male (5.9%) camels. There were no sex and age linked seroreactivity differences ($p < 0.05$)

Dubey *et al,* (2006) studied that Neospora caninum, *Sarcocystis* spp, and *Toxoplasma gondii* are related coccidian parasites that can cause abortion and neonatal mortality in animals. In addition, T gondii and certain species of Sarcocystis are zoonotic. This article reviews information on the etiology, diagnosis, control, and prevention of these diseases

Innes *et al,* (2007) it is sometimes easy to make the mistake of assuming that everything that holds true for Toxoplasma gondii is also true for its relative Neospora caninum. However, a recurring theme in the recent review by Hemphill et al. is not the similarities but the striking differences between the two parasites.

MATERIAL & METHODS

CHAPTER 3

MATERIAL AND METHODS

Experimental site:

The study was conducted at various camel colonies of Bahawalpur and Department of Parasitology, University of Veterinary & Animal Sciences, Lahore.

Collection of samples:

A total of 100 blood samples of camels were collected at random from various camel colonies of Bahawalpur. The record/history of each animal was recorded in performa. Under aseptic measures, 5-10 ml of blood was drawn from each camel by vein puncture with the help of disposable syringes and was transferred to screw capped sterile test tube, slowly to avoid haemolysis.

Separation of serum:

All the blood samples were labelled with number and date of collection. The samples were left for about an hour for blood clotting to occur. The clotted blood was then separated with the help of a fine loop and blood samples were centrifuged at 3500 rpm for at least 5 minutes. The supernatant clean sterile blood was aspirated with a pasture pipette and transferred into a screw capped vial which was stored at –20 C°degree until processed for analysis.

Analysis of serum samples:

All the serum samples were analyzed for *Toxoplasma* specific IgG antibodies using Latex Agglutination, (LA) test. For this purpose, the commercial Toxoplasma Latex test kit was used.

Reagent and Controls:

The commercial test kit for Toxoplasma latex tests contains the following contents.

1 x 4.0ml Latex reagent

1 x 0.5ml positive control

1 x 0.5ml negative control slide-4

Disposable Stirrers

Storage and stability of Toxoplasma Latex Kit:

All the components were stored between 2-8 C to maintain the stability of the contents according to the manufacturer's recommendations.

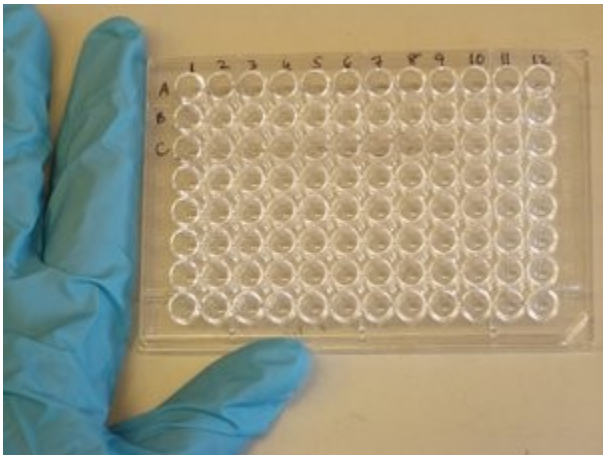
Technique:

The Toxoplasma kit was used according to the manufacturer's instructions.

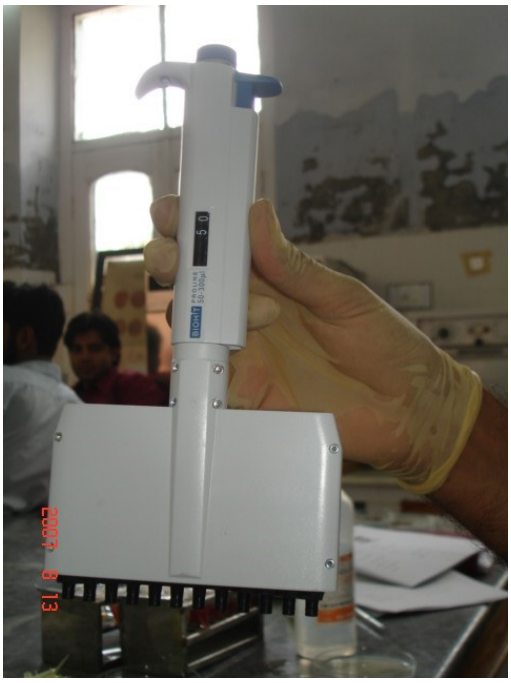
1. Prior to running the assay, all test reagent, was brought to average room temperature (20 C°) and sera was thawed.
2. 25 microlitre of physiological normal saline (0.9% Nacl) solution (N.S.S) was added to each well of 96 microlitre plate with the help of micro dispenser. The 25 microlitre of the test sera was added to the first well. This was mixed with N.S.S and 25 microlitre from this was then be transferred to the adjacent well and so on. 25

microlitre of the mixture was discarded from the last well. In this serial, dilutions was made by two fold dilution technique to 8th well 1:256.

3. One drop of diluted serum each from1:16, 1:128, and 1:256 dilutions was placed on to a slide.
4. The latex reagent was mixed well and one drop of reagent was added over each Serum drop.
5. Both drops were mixed well with the aid of a disposable stirrer and slide was tilted slightly.
6. The presence or absence of agglutination was observed within a period no longer than five minutes.
7. The antibody titre was shown by the significant agglutination in different dilution of the test serum.



Micro Titer plate



Micro Titer pipette

The Toxoplasma Latex Kit

Interpretation of Results:

The following interpretation was made:

Negative: 1:16 sera dilution indicates absence of immunity

Positive: 1:16 Sera dilution was indicating residual or non specific immunity.

Positive: titre from 1:32 to 1:128 depicts acquired or evolving immunity.

Positive: titre equal or higher than 1:256 suggest possible recent contact

Seroprevalance:

The data was analyzed at different angles by calculating the Percentage of seropositivity of *Toxoplasma gondii*.

Dispensing of Serum Samples and Latex Reagent

Stirring of serum sample and Latex Reagent

RESULTS

CHAPTER 4
RESULTS

Prevalence of *Toxoplasmosis* in Camels:

A total 100 blood samples of camels were collected and analyzed for anti-toxoplasma antibodies at screening dilution of 1:16, 1:128,1:256 by using commercially available Toxoplasma Latex Kit on the principle of Latex Agglutination Test (LAT).

The camels age ranged from 1-15years & above blood samples were taken and divided in to 3 categories i.e. A-1, A-2, A-3. Twenty samples were taken from age (A-1) ranged between 1-5 years, 36 samples were taken from age (A-2) ranged between 6-10 years and 44 samples were taken from age (A-3) ranged between 11-15 years & above (Table 1).The age categories (A-2) ranged from 6-10year had the highest seropositive percentage that was 16.6 % followed by A-3 (11-15 yr & above) that was 9.0 %, where as the number of samples tested in A-1 (1-5 yr) had no positive case (Table 1 & Figure 1).

As for as the sex of camels was concerned, 46 were male and 54 female (Table 2). Female camels have higher seropositive percentage (11.1 %) than male (8.69 %) (Figure 2).

The overall seropositive percentage was 10%. According to antibodies titer, 2 camels showed antibody titer at screening dilution of 1:16, 5 camels showed antibody titer at 1:128 and 3 showed antibody titer at screening dilution of 1:256 (Table1).

Table 1. Overall Prevalence of *Toxoplasma gondii* antibodies in Camels by using Latex Agglutination Test (LAT) in relation to their Age.

Age (Years)	No of sera Tested	Antibodies Titer			Seropositive	% Seropositive
		1:16	1:128	1:256		
A-1 (1-5)	20	0	0	0	0	0
A-2 (6-10)	36	01	03	02	06	16.6 %
A-3 (11-15) & above	44	01	02	01	04	9.0 %
Total	100	02	05	03	10	10 %

A= Age

Figure 1. Overall Prevalence of *Toxoplasma* antibodies in Camels by using Latex Agglutination Test (LAT) in relation to their Age.

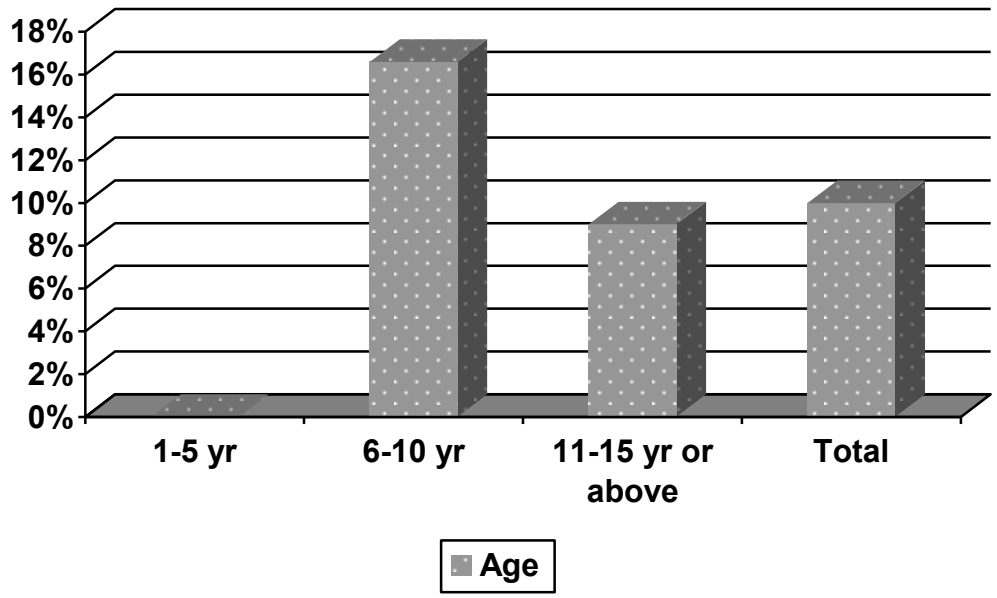
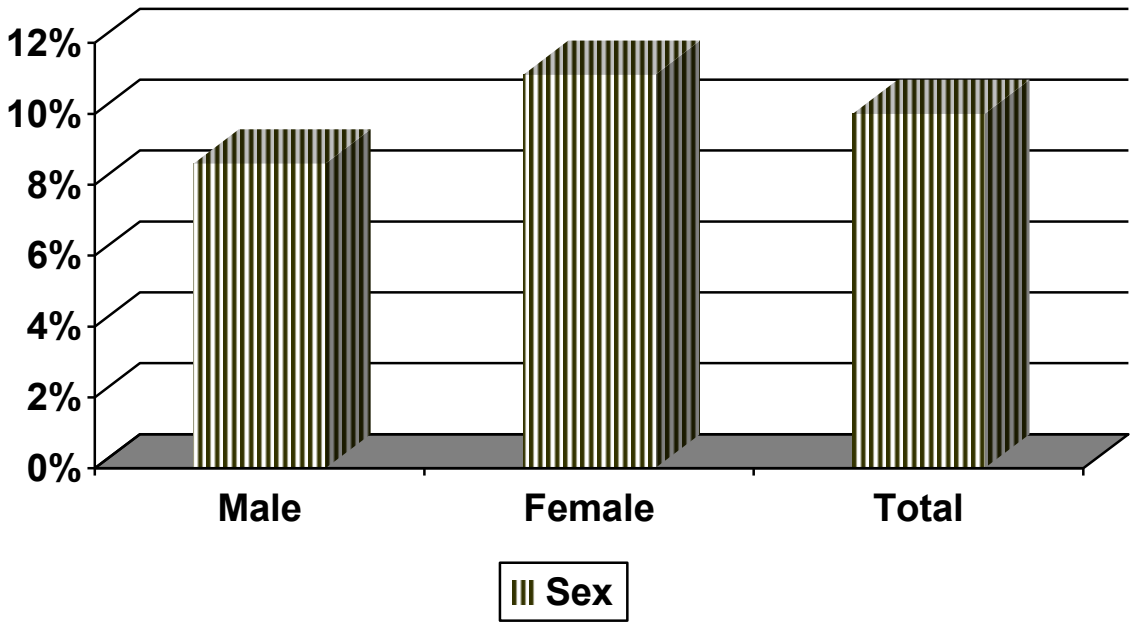
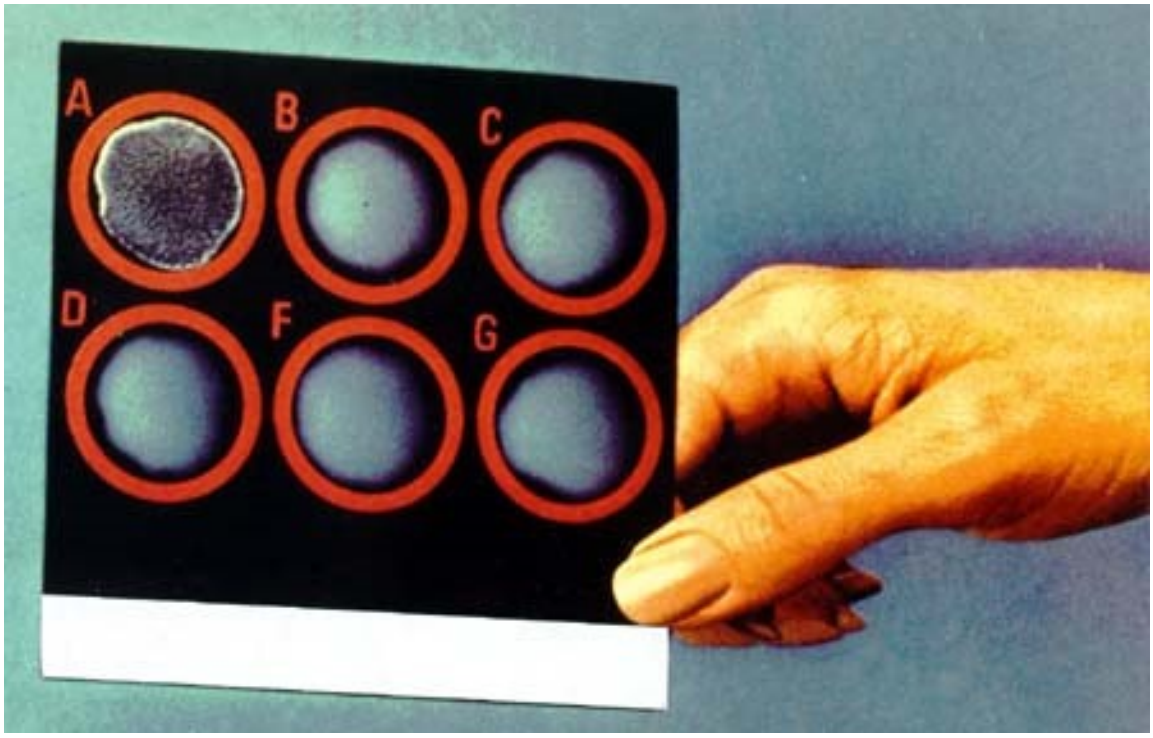


Table 2. Overall Prevalence of *Toxoplasma gondii* antibodies in Camels by using Latex Agglutination Test (LAT) in relation to their Sex.

Sex	No of sera Tested	Antibodies Titer			Seropositive	% Seropositive
		1:16	1:128	1:256		
Male	46	01	02	01	04	8.69 %
Female	54	01	03	02	06	11.1 %
Total	100	02	05	03	10	10 %

Figure 2. Overall Prevalence of *Toxoplasma* antibodies in Camels by using Latex Agglutination Test (LAT) in relation to their Sex.





Agglutination of Latex Reagent with Antibodies
at screening dilution of 1:16, 1:128, 1: 256

DISCUSSION

CHAPTER 5

DISCUSSION

Toxoplasmosis is one of the most common infection in men and animals cause by protozoan parasite *T. gondii,* which is responsible for significantly higher morbidity, and mortality in both human and other warm-blooded animals. Toxoplasmosis is world wide distribution, zoonotic in nature and depending upon the geographic location. 15-85% of the population can be symptomatically infected (Subash 1990). *Toxoplasmosis* is also responsible for abortion and congenital defects in human and domestic livestock including sheeps, goats, camels, cows and buffaloes (Pedro *et, al.,* 2003).

T.gondii can cause severe acquired and congenital infection in animals as well as human beings associated with fever, lymphadenitis, uveitis, muscle fatigue, hepatitis, encephalitis and abortion. Serological surveys indicate that about 80 % of all primary infections are asymptomatic, due to the immune system effectiveness, but variable levels of the disease can affect immunocompromised individuals (Cantos *et. al.,* 2000). The acquired immune deficiency syndrome (AIDS) has created an expanding population of susceptible individuals. Usually people suffering from both AIDS and *Toxoplasmosis* have been exposed to the *Toxoplasma* parasite earlier in life and the HIV infection simply allowed the *Toxoplasma* parasite to grow unchecked. The concomitant occurrences should be considered by public health policies especially in those countries with high Toxoplasmosis prevalence, where AIDS is concurrent with economic and public health

problems (Passos *et. al.,* 2000). *Toxoplasma gondii* infection is embrotoxic in humans as well as animals. It is mainly transmitted through raw or undercooked meat and ingestion of oocysts in cat feces.

Toxoplasmosis in camels is important because of its zoonotic importance and camels are main source of meat consumption in Pakistan. Due to the zoonotic importance of toxoplasmosis, the present study was conducted to sort out the seroprevalence of *T. gondii* infection in camels in & around Bahawalpur areas by using commercially available Toxoplasma Latex Agglutination kit (LAT). In this study 100 blood samples of camels were collected from camel colonies at Bahawalpur. Serum was separated after centrifugation. The data about the age and any disease history was noted and the results thus obtained were recorded in separate performa.

Among 100 camels examined in the present study, 2 gave an antibody titer of 1:16 which indicated residual or non specific immunity, 5 gave antibody titer of 1:128 which was due to acquired or evolving immunity, where as three camels were positive at antibody titer of 1:256 strongly suggested present infections as reported by (Fanck *et. al.,* 2004).

The overall prevalence of anti toxoplasma antibodies in camels was recorded as 10 %. *Toxoplasma gondii* antibodies are widly spread in animal's population, which supported that *toxoplasmosis*, is widely spread zoonotic infection (Mirdha *et, al.,* 1999). Various researchers recorded the prevalence of anti *toxoplasma* antibodies in camels using different serological tests including Latex agglutination test (LAT) by Chaudhary *et al,* (1996) at Abu-Dhabi (18%), Abu-Zeid (2002) at Abu-Dhabi (31.4%), Khalil *et al,* (2002) at Sudan (22.2%), Hilali *et al,* (1998) at Egypt (17.4%), Afzal *et al,* (1994) at

Abu-Dhabi (30.9%) Elamin *e . al,* (1991) at Sudan (67%). Indirect Fluorescent antibodies test (IFT) i.e. A. Sadrebazzaz *et al,* (2005) in Iran (4.16%), Karimi (2006) in Iran (6%). Indirect Haemagglutination test (IHAT) . Hussein MF *et al,* (1998) in Saudi Arabia (16%), Ibrahim *et al,* (1997) in Egypt (44.1%), Yousif *et al,* (2005) in Abu-Dhabi (22.4%). The variation in seroprevalence results of *Toxoplasmosis* in camels in different part of the world was due to difference in environmental and managemental conditions in various geographical areas.

The seroprevalence of *T.gondii* in camels varied with age. The highest (16.6%) seropositive percentage was found in A-2 (6-10 yr) followed by (9.0%) A-3 (10-15 yr & above) and no seropositive case was recorded in A-1 (1-5 yr). These findings are in concomitant with the results of Gualterbo Marcas *et al,* (2004), Elamin *et al,* (1991), Karimi (2006).

As far as sex of camels was concerned, female camels have the higher seropositive percentage i.e. 11.1% most of them having a history of abortion followed by male camels i.e. 8.69%. The present study revealed that the prevalence of anti toxoplasma antibodies more in female camels than male was in concomitant with the results of Hussein MF *et. al.,*(1998).

From the current data, it was concluded that prevalence of *Toxoplasmosis* in camels was 10%. Camels in group A2 from 6-10 years were found to be more commonly affected than other groups and infection was higher in females than males.

The prevalence of toxoplasma infection in humans and animals is often associated with infection in pets. Little attention however has been given to domestic pet despite their intimate contact with animals and their feed. Toxoplasma is a true zoonosis occurring in man, domestic and wild animals and birds. Although only a preliminary study showed that the chances of contacting toxoplasma, through the ingestion of oocyst is very high. Keeping in view of these findings, following preventive measures should be adopted to reduce the exposure rates.

Recommendations:

TO REDUCE EXPOSURE IN CAMELS

1) Pets especially cat should be kept away from animal feed and grazing areas to avoid contamination by cat faeces.
2) Pets' litter should not be disposed off in yard or grazing areas.
3) Camels should be fed only clean and hygienic feed.
4) Proper screening at regular interval must be done

SUMMARY

CHAPTER 6
SUMMARY

Toxoplasma gondii is an intracellular parasite, which infects humans and animals by ingestion of tissue cyst , raw or undercooked meat or oocysts from soil, vegetables, fruits, water, soil and food contaminated by cat faeces or by transmission through the placenta, milk and blood transfusion. Although toxoplasmosis found throughout the world. Seropositivity levels vary widely among different regions of the globe and according to sociocultural habits, geographic factors; climate and transmission routes and typically rise with age.

In present study, the overall prevalence of *T. gondii* infection in camels at Bahwawlpur recorded as 10% by using Toxoplasma Latex Agglutination kit (LAT). 2 camels were found seropositive at 1:16 dilution showing residual or non specific immunity, 5 camels were found seropositive at 1:128 showing acquired or evolving immunity, whereas 3 camels were positive at antibody titer of 1:256 giving an evidence of present infection.

It was also noted that seropositivity of *T. gondii* in camels was higher in age group from 6-10 years, infection was higher in female camels having abortion history than male.

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CHAPTER 7
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